

AMENDMENTS TO THE SPECIFICATION

Please replace Paragraphs [0048] and [0049] with the following paragraph rewritten in amendment format:

[0043] Figure 6 illustrates a detailed arrangement of components *in planta*. (a) illustrates the antisense relationship of the gene and IRES (SEQ ID NOs:16 & 17) with respect to the promoter and the viral 3' UTR. Upside down nucleotides indicate antisense orientation. (b) illustrates the RNA polymerase transcript with the gene and IRES (SEQ ID NO:18) in upside down antisense orientation. (c) illustrates final conversion resulting in both the IRES and gene (SEQ ID NO:19) in a translatable orientation. AATTC indicates IRES; ATG indicates initiation codon; XXX indicates any codon; YYY indicates complements of a codon; asterisk indicates a stop codon.

[0048] In the DNA sequence comprising an anti-sense coding sequence for a heterologous polypeptide, non-limiting examples of the heterologous polypeptide encoded by the complement of the anti-sense coding sequence, include, for illustrative purposes only, hormones and hormone precursors, such as, for example, thyroid-stimulating hormone, follicle-stimulating hormone, luteinizing hormone, prolactin, growth hormone, adrenocorticotrophic hormone, growth hormone-releasing hormone, corticotropin-releasing hormone, somatostatin, calcitonin, parathyroid hormone, human chorionic gonadotropin, insulin, glucagon, somatostatin, erythropoietin, atrial-natriuretic peptide, gastrin, secretin, cholecystokinin, somatostatin, neuropeptides, insulin-like growth factor-1, angiotensinogen, thrombopoietin and leptin; enzymes, such as, for example, oxidoreductases such as, for example, dehydrogenases, oxidases, reductases and catalases; transferases such as, for example, acetyltransferases, methylases, protein kinases and phosphatases; hydrolases including proteases, nucleases and phosphatases such as, for example, alkaline phosphatase or phytase; lyases including decarboxylases and aldolases; isomerases, such as, for example, epimerases and racemases; and ligases such as, for example, peptide synthases, aminoacyl-tRNA synthetases, DNA ligases and RNA ligases; cell toxins such as, for example, barnase; cell surface proteins such as, for example, transport proteins and receptor proteins; intracellular proteins such as, for example, proteins associated with intracellular signaling such as G-proteins and associated receptors, proteins associated with intracellular transport; structural

proteins; reporter proteins such as, for example, beta-galactosidase and fluorescent proteins such as a green fluorescent protein; proteins conferring disease resistance, such as, for example, a viral coat protein polypeptide; antibodies, such as, for example, a "plantibody" (Gibbs, WW. *Scientific American* 277: 44, 1997), and numerous other proteins and polypeptides. The polypeptide can comprise, for example, a naturally occurring amino acid sequence, or conservative amino acid substitutions, deletions, or additions thereof which do not destroy the polypeptide's activity. Thus, the polypeptide can also comprise additional sequences, such as, for example, a leader sequence for cell secretion; a target sequence for a biotinylation reaction catalyzed by a biotin ligase; a polyhistidine sequence for purification on a heavy metal ion column such as, for example, a zinc ion column; an epitope tag, such as, for example, a FLAG sequence or a myc epitope tag; and a protease recognition site, such as, for example, an enterokinase recognition site. The DNA sequence comprising an anti-sense coding sequence for a heterologous polypeptide can comprise an artificial sequence or a naturally occurring DNA sequence. A DNA sequence encoding a polypeptide can encode translation codons that reflect the preferred codon usage of a host cell or organism. For example, if the host cell or organism species is *Nicotiana benthamiana*, a codon usage table ~~such as that published on the internet at <http://www.kazusa.or.jp/codon/cgi-bin/showcodon.cgi?species=Nicotiana+benthamiana+gbp1n>~~ can be used to select codons or their complements in designing an artificial DNA sequence or modifying a naturally occurring DNA sequence. It is expected that use of preferred codons in a coding sequence will lead to higher efficiency of translation of a transgene in a transgenic cell or organism. The DNA sequence comprising an antisense coding sequence for a heterologous polypeptide can further comprise one or more antisense introns, at least one antisense translation termination codon, and a transcription termination signal.

[0049] The DNA sequence complementary to an IRES can comprise a sequence complementary to any known IRES. The IRES can be, for example, any IRES known in the art to function to support internal ribosomal entry of an RNA in a eukaryotic cell. The IRES, therefore, may derive from any number of different viruses, animals, plants, or eukaryotic microorganisms, or may be an artificial IRES. Non-limiting examples of an IRES that can be used in the invention include those retrievable from an internet database (Bonnal et al., *Nucleic*

Acids Res. 31: 427-428, 2003). Non-limiting examples of an IRES include a picornavirus IRES (Jang et al, *Enzyme* 44: 292-309, 1990; Roberts et al., *RNA* 4: 520-529, 1998), a foot-and-mouth disease virus IRES (Kuhn et al., *J. Virol.* 64: 4625-4631, 1990); an encephalomyocarditis virus IRES (Evstafieva et al., *Nucleic Acids Res.* 19: 665-671, 1991), a hepatitis A virus IRES (Brown et al., *J. Virol.* 65: 5828-5838, 1991), a hepatitis C virus IRES (Tsukiyama-Kohara et al., *J. Virol.* 66: 1476-1483, 1992), a human rhinovirus IRES (Borman et al., *Virology* 188: 685-696, 1992), a poliovirus IRES (Haller et al, *J. Virol.* 66: 5075-5086, 1992; Klinck et al., *Nucleic Acids Res.* 25: 2129-2137, 1997), a swine vesicular disease virus IRES (Chen et al., *J. Virol.* 67: 2142-2148, 1993), a turnip mosaic potyvirus IRES (Basso et al., *J. Gen. Virol.* 75: 3157-3165, 1994), a human fibroblast growth factor 2 mRNA IRES (Vagner et al., *Mol. Cell Biol.* 15: 35-44, 1995), a pestivirus IRES (Poole et al., *Virology* 206: 750-754, 1995), a Leishmania RNA virus IRES (Maga et al., *Mol. Cell Biol.* 15: 4884-4889, 1995), a Moloney murine leukemia virus IRES (Vagner S, *J. Biol. Chem.* 270: 20376-20383, 1995), a human rhinovirus 14 IRES (Rojas-Eisenring et al., *J. Virol.* 1995 69: 6819-6824, 1995), an aphthovirus IRES (Martinez-Salas et al., *J. Virol.* 70: 992-998, 1996), a human immunoglobulin heavy chain binding protein (BiP) mRNA IRES (Le et al., *Nucleic Acids Res.* 25: 362-369, 1997), a *Drosophila* Antennapedia mRNA IRES (Le et al., *Nucleic Acids Res.* 25: 362-369, 1997), a human fibroblast growth factor 2 (FGF-2) mRNA IRES (Le et al., *Nucleic Acids Res.* 25: 362-369, 1997), a hepatitis G virus IRES (Pickering et al., *J. Viral. Hepat.* 4: 175-184, 1997), a tobamovirus IRES (Ivanov et al., *Virology* 232: 32-43, 1997), a vascular endothelial growth factor mRNA IRES (Stein et al., *Mol. Cell Biol.* 18: 3112-3119, 1998), a Coxsackie B group virus IRES (Carthy et al., *Clin. Exp. Pharmacol. Physiol.* 24: 997-1003, 1997), a c-myc protooncogene mRNA IRES (Nanbru et al., *J. Biol. Chem.* 272: 32061-32066, 1997; Nanbru et al., *Oncogene* 20:4270-4280, 2001), a human MYT2 mRNA IRES (Kim et al., *Mol. Cell Neurosci.* 12:119-140, 1998), a human parechovirus type 1 virus IRES (Ghazi et al., *J. Gen. Virol.* 79: 2641-2650, 1998), a human parechovirus type 2 virus IRES (Ghazi et al., *J. Gen. Virol.* 79: 2641-2650, 1998), a eukaryotic initiation factor 4GI mRNA IRES (Johannes et al., *RNA* 4: 1500-1513, 1998), a *Plautia stali* intestine virus IRES (Sasaki et al., *J. Virol.* 73: 1219-1226, 1999), a Theiler's murine encephalomyelitis virus IRES (Yamasaki et al., *J. Virol.* 73: 8519-8526, 1999), a bovine enterovirus IRES (Zell et al., *J. Gen. Virol.* 80: 2299-2309, 1999), a connexin 43 mRNA IRES (Schiavi et al., *FEBS Lett.* 464: 118-122, 1999), a homeodomain protein Gtx mRNA IRES (Chappell et al., *Proc. Natl. Acad. Sci.*

USA 97: 1536-1541, 2000), an AML1 transcription factor mRNA IRES (Pozner et al., *Mol. Cell. Biol.* 20: 2297-2307, 2000), an NF-kappa B repressing factor mRNA IRES (Oumard et al., *Mol. Cell. Biol.* 20: 2755-2759, 2000), an X-linked inhibitor of apoptosis (XIAP) mRNA IRES (Holcik et al., *Mol. Cell Biol.* 20: 4648-4657, 2000), a cricket paralysis virus RNA IRES (Wilson et al., *Mol. Cell Biol.* 20: 4990-4999, 2000), a p58(PITSLRE) protein kinase mRNA IRES (Cornelis et al. *Mol. Cell* 5: 597-605, 2000), an ornithine decarboxylase mRNA IRES (Pyrnnet et al., *Mol. Cell* 5: 607-616, 2000), a connexin-32 mRNA IRES (Huddner et al., *J. Biol. Chem.* 275: 34586-34591, 2000), a bovine viral diarrhea virus IRES (Sanderbrand et al., *Vet. Microbiol.* 77: 215-227, 2000), an insulin-like growth factor I receptor mRNA IRES (Giraud et al., *J. Biol. Chem.* 276: 5668-5675, 2001), a human immunodeficiency virus type 1 gag gene IRES (Buck et al., *J. Virol.* 75: 181-191, 2001), a classical swine fever virus IRES (Kolupaeva et al., *RNA* 6: 1791-1807, 2000), a Kaposi's sarcoma-associated herpesvirus IRES (Grundhoff et al., *J. Virol.* 75: 1857-1863), a short IRES selected from libraries of random oligonucleotides (Owens et al., *Proc. Natl. Acad. Sci. USA* 98: 1471-1476, 2001), 2001; Bielecki et al., *J. Virol.* 75: 1864-1869, 2001), a Jembrana disease virus IRES (Metharom et al., *Vet. Microbiol.* 80: 9-22, 2001), an apoptotic protease-activating factor 1 mRNA IRES (Mitchell et al., *Mol. Cell Biol.* 21: 3364-3374, 2001), a Rhopalosiphum padi virus IRES (Woolaway et al., *J. Virol.* 75: 10244-10249, 2001), a cationic amino acid transporter mRNA IRES (Fernandez et al., *J. Biol. Chem.* 277: 11780-11787, 2002), a human insulin-like growth factor II leader 2 mRNA IRES (Pedersen et al., *Biochem. J.* 363: 37-44, 2002), a giardiavirus IRES (Garlapati et al., *RNA* 8: 601-611, 2002), a Smad5 mRNA IRES (Shiroki et al., *Nucleic Acids Res.* 30: 2851-2861, 2002), a porcine teschovirus-1 talfan IRES (Kaku et al., *J. Virol.* 76: 11721-11728, 2002), a *Drosophila* Hairless mRNA IRES (Maier et al., *Proc. Natl. Acad. Sci. USA* 99:15480-15485, 2002), an hSNM1 mRNA IRES (Zhang et al., *DNA Repair (Amst)* 1: 379-390, 2002), a Cbfa1/Runx2 mRNA IRES (Xiao et al., *J. Cell Biochem.* 88: 493-505, 2003), an Epstein-Barr virus IRES (Isaksson et al., *Oncogene* 22: 572-581, 2003), a hibiscus chlorotic ringspot virus IRES (Koh et al., *J. Biol. Chem. in press*), published on the internet at <http://www.jbc.org/cgi/reprint/M210212200v1.pdf>, a rat pituitary vasopressin V1b receptor mRNA IRES (Aguilera et al., *J. Mol. Endocrinol.* 30: 99-108, 2003), and a human hsp70 mRNA IRES (Rubtsova et al, *J. Biol. Chem. in press*), available on the internet at <http://www.jbc.org/cgi/reprint/M303213200v1.pdf>.